Development and Evaluation of a Mini Core Collection for the U.S. Peanut Germplasm Collection

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ABSTRACT

A core collection (831 accessions) has been developed to represent the U.S. Arachis hypogaea L. germplasm collection. This core collection has been shown to be effective in improving the efficiency of identifying genes of interest in the entire germplasm collection. However, an even smaller subset of germplasm is needed for traits which are difficult and/or expensive to measure. The objectives of this study were to select a core of the core collection and to evaluate the usefulness of this subset of germplasm to identify genes of interest in peanut. Data for eight above ground and eight below ground morphological characteristics were measured for each accession in the core collection. Cluster analysis was used on these data to partition the core accessions into groups which, theoretically, are genetically similar. Random sampling was then used to select a 10% sample from each group. The result was a core of the core collection (mini core) consisting of 112 accessions. Examination of morphological data indicated that the majority of genetic variation expressed in the core collection has been preserved in this core of the core collection. Data on disease resistances for accessions in the core collection were collected and used to determine retrospectively how effective the use of a mini core collection would have been in identifying sources of resistance in the core collection. Results indicated that the mini core collection can be used to improve the efficiency of identifying desirable traits in the core collection. For resistance to late leaf spot (Cercosporidium personatum (Berk. & M. A. Curtis), the use of a mini core collection would have improved the efficiency of identifying resistant accessions in the entire collection. The core of the core approach should be particularly useful for traits which are difficult and/or expensive to measure.

Nore EFFICIENT METHODS for evaluating and maintaining genetic diversity in germplasm collections are needed. One possible method is the development and use of core collections (Frankel, 1984; Frankel and Brown, 1984). A core collection has been developed for the U.S. peanut germplasm collection (Holbrook et al., 1993). Data on peanut in the Germplasm Resource Information Network (GRIN) were used to select this core collection. The U.S. germplasm collection was first stratified by country of origin and then divided into nine sets based on the amount of additional information available for accessions and on the number of accessions per country of origin. This procedure resulted in the selection of 831 accessions from the U.S. *A hypogaea* germplasm collection.

The core collection approach to germplasm evaluation is a two-stage approach. The first stage involves

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Published in Crop Sci. 45:1540–1544 (2005). Plant Genetic Resources doi:10.2135/cropsci2004.0368 © Crop Science Society of America 677 S. Segoe Rd., Madison, WI 53711 USA examining all accessions in the core collection for a desired characteristic. This information is then used to determine which clusters of accessions in the entire germplasm collection should be examined during the second stage of screening. Theoretically, the probability of finding additional accessions with a desired characteristic would be highest in these clusters. Holbrook and Anderson (1995) used data on resistance to late leaf spot that was available for the entire peanut germplasm collection to determine retrospectively how effective the use of a core collection approach would have been for identifying sources of resistance in the entire collection. Holbrook et al. (2000b) evaluated the effectiveness of a two-stage core screening approach in identifying resistance to the peanut root-knot nematode [Meloidogyne arenaria (Neal) Chitwood race 1] in the U.S. germplasm collection of peanut. Results from both of these studies demonstrated that the peanut core collection can be used to improve the efficiency of identifying genes of interest in the entire germplasm collection.

The peanut core collection has been very effective in enhancing the utilization of peanut genetic resources (Holbrook, 1999). However, an even smaller subset of germplasm is needed for traits which are difficult and/or expensive to measure. Upadhyaya and Ortiz (2001) suggested a strategy for sampling the entire and core collections for developing a mini core subset which contains about 1% of total accessions in the entire collection but captures most of the useful variation of the crop. A peanut mini core collection has been selected (Upadhyaya et al., 2002) from the ICRISAT core collection (Upadhyaya et al., 2003). The objectives of this study were to develop a core of the core collection (mini core) for the U.S. peanut core collection and to use information on resistances to leaf spot, Tomato spotted wilt virus (TSWV), aflatoxin, and root-knot nematode for the core collection accessions to evaluate the core of core concept.

MATERIALS AND METHODS

Selection of a Core of the Core Collection

Data for eight above-ground and eight below-ground morphological characteristics (Pittman, 1995) were measured for each accession in the core collection. The eight above-ground descriptors were growth habit, plant size, prominence of main stem at mid season, prominence of main stem at harvest, presence of flowers on the main axis, leaf color, stem pigmentation, and maturity. The below-ground descriptors were measured post harvest and included pod shape, pod constriction, pod reticulation, seed per pod, 100-pod weight, U.S. pod market type, seed coat color, and 100-seed weight.

Abbreviations: GRIN, germplasm resources information network; TSWV, *Tomato spotted wilt virus*.

Cluster analysis was used on this data set to partition the core accessions into groups which theoretically, are genetically similar. The cluster procedure (SAS Institute, 2000) with Ward's minimum variance cluster analysis was used to divide accessions into an appropriate number of clusters. The tree procedure (SAS Institute, 2000) was used to print the clusters. Random sampling was then used to select a 10% sample resulting in a core of the core collection (mini core) consisting of 112 accessions.

Evaluation of Core Collection Accessions for Disease and Pest Resistances

Peanut Root-Knot Nematode

Resistance was evaluated in greenhouse studies at Tifton, GA, by the screening technique described by Holbrook et al. (1983) with five replications (five plants). Plants were grown in stream-pasteurized loamy sand (85% sand, 11% silt, 4% clay). Each plant was inoculated with 3500 eggs of *M. arenaria* race 1, which had been cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Nematode inoculum was prepared by the NaOCl method (Hussey and Barker, 1973) and applied 10 d after planting.

Plants were uprooted and washed clean of soil 90 d after inoculation. The roots were placed in 1000-mL beakers containing 300 mL of phloxine B solution for 3 to 5 min (Daykin and Hussey, 1985). Each plant was indexed for root galls and egg masses on the following scale: 0 = no galls or no egg masses, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, 5 = more than 100 galls or egg masses per root system (Taylor and Sasser, 1978). Accessions were considered resistant if they exhibited a mean egg mass rating less than or equal to 2.5.

Leaf Spot

Resistance to leaf spot was evaluated in field plots in Tifton, GA. Natural disease pressure was a combination of early (Cercospora arachidicola Hori) and late (Cercosporidium personatum Berk. & M. A. Curtis) leaf spot. The core collection was divided into five sets of entries with similar maturities. Sets were planted in a randomized complete block design with two replications. Seeds of accessions were planted in two-row plots, 2 m long. Standard cultural practices for peanut were followed with the exception that no fungicides were used for leaf spot control. Natural incidence of late leaf spot was heavy. Disease severity was evaluated on all plots with the 1-to-10 Florida leaf spot disease rating scale (Chiteka et al., 1988; Knauft et al., 1988) in which 1 = no leaf spot and 10 = total plant death due to leaf spot. Accessions were considered resistant if they exhibited a mean rating less than or equal to 6.0.

Tomato spotted wilt virus

Resistance to TSWV was evaluated in field plots at Tifton and Attapulgus, GA. The core collection was divided into five sets of entries with similar maturities. Sets were planted in a randomized complete block design with two replications. Seeds of accessions were planted in two-row plots, 2 m long. Standard cultural practices for peanut production were followed, and the natural incidence of TSWV was heavy. Spotted wilt intensity was evaluated in each plot by a disease intensity rating that represents a combination of incidence and severity (Culbreath et al., 1997). The number of 0.3-m portions of row containing severely stunted, chlorotic, wilted, or dead plants was counted for each plot and then converted to a percentage

of row length. Accessions were considered resistant if they exhibited a mean rating of less than or equal to 40%

Aflatoxin

Resistance to aflatoxin contamination was evaluated after imposing heat and drought stress on field plots at Tifton, GA. Groups of 20 genotypes with similar maturities were planted in a randomized complete block design with five replications. Seeds were planted in single-row plots, 1.5 m long at six seeds/ 30 cm linear row. Inoculum of Aspergillus flavus Link ex Fries (NRRL 3357) and A. parasiticus Speare (NRRL 2999) was prepared (Will et al., 1994) and introduced into test plots to ensure the presence of sufficient aflatoxin-producing fungi in the peanut pod zone. Aflatoxin contamination was measured by the immunoaffinity column fluorometer method (Trucksess et al., 1991). Relative toxin concentration was calculated to attempt to standardize for the large amount of naturally occurring test-to-test variability, where relative toxin = entry mean toxin/test mean toxin. Accessions were considered resistant if they exhibited a mean relative toxin less than or equal to 0.4.

Statistical Analysis to Evaluate the Core of the Core Collection

Disease ratings for the core of the core collection accession(s) within each cluster were defined as the indicator values for that cluster. All accessions in clusters with resistant indicator values were included in the hypothetical second-stage screening. Data were visually examined to determine how many disease resistant accessions would have been identified by examining the core of the core collection. Data were also examined to determine how many disease resistant accessions would have been identified by examining all accessions from clusters having a resistant indicator value (second-stage screening). Success rates [(number of resistant accessions identified/total number of accessions screened) \times 100] were calculated for various subsets of the entire collection. Comparisons of success rates were made by calculating chi-square values with observed and expected numbers of resistant and susceptible accessions. The Yates correction term was used since this adds to the accuracy of chi-square analysis when the number of an expected class is small (Strickberger, 1976).

RESULTS AND DISCUSSION

The selection of a core collection for the U.S. germ-plasm collection of peanut has helped accelerate work on germplasm evaluation and has resulted in the identification of numerous genes that will be of value in cultivar development (Holbrook and Stalker, 2003). The development of a core of the core collection (mini core) should result in additional genetic information on peanut. The first step toward this goal was the development of a data set containing measurements for 16 morphological characteristics for all accessions in the core collection. Cluster analysis of these data resulted in the grouping of the core accessions into 26 clusters. A 10% random sample resulted in the selection of 112 genotypes to form the core of the core collection.

The means for 11 morphological characteristics and for three disease resistances for this core of the core

Table 1. Means for eleven morphological variables and three disease resistances for the U.S. peanut core collection and for the core of the core collection and the range of these variables in the core of the core collection expressed as a percentage of the range of the core collection.

Variable†		Core of core collection		
	Core collection		Range of	
	Mean	Mean	core collection	
Growth habit	4.4	4.4	100	
Plant size	3.5	3.5	100	
Leaf color	2.8	3.1	100	
Maturity	2.6	2.8	100	
Pod shape	4.0	4.0	100	
Pod constriction	3.6	3.7	100	
Pod reticulation	2.9	2.9	100	
Pod weight	109	110	70	
Market type	3.3	3.3	100	
Seed weight	46	47	72	
Shell out	70	70	73	
Egg mass	4.0	4.1	81	
TŠWV	64	65	75	
Leaf spot	7.4	7.1	92	

† Growth habit: 1 = Prostate; 2 = Spreading; 3 = Spreading and bunch; 4 = Bunch; 5 = Erect; 6 = Mixed.

Plant size: 1 = Dwarf; 2 = Small; 3 = Medium; 4 = Large; 5 = Extra large; 6 = Mixed.

Leaf color: 1 = Very light green; 2 = Light green; 3 = Green; 4 = Dark green; 5 = Very dark green; 6 = Mixed; 7 = Other colors.

Maturity: 1 = Very early; 2 = Early; 3 = Medium; 4 = Late; 5 = Very

late: 6 = Mixed.

Pod shape: 1 = Vulgaris; 2 = Fastigiata; 3 = Peruviana; 4 = Hypogaea; 5 = Hirsuta; 6 = Mixed; 7 = Other.

Pod constriction: 0 = None; 1 = Slight; 2 = Moderate; 3 = Deep; 4 =

Very deep; 5 = Mixed; 6 = Other. Pod reticulation: 1 = Smooth; 2 = Slight; 3 = Moderate; 4 = Rough; 5 = Mixed.

Pod weight: g per 100 mature pods.

Market type: 1 = Spanish; 2 = Valencia; 3 = Runner; 4 = Virginia; 5 = Mixed.

Seed weight: g per 100 mature seeds.

Shell out: g of kernels per 100 g of pods.

Egg mass: 0 = no nematode egg-masses; 1 = 1 to 2; 2 = 3 to 10; 3 = 11to 30; 4 = 31 to 100; 5 = more than 100 egg-masses.

TSWV: Percentage of the total row length with plants severely affected

Leaf spot: Florida disease rating scale where 1 = no disease and 10 = no

and for the complete core collection were similar (Table 1). In addition, the range for the each of the variable in the mini core included at least 70% of the range exhibited in the complete core collection. This suggests that this subset of the core collection is a representative sample of the core collection and that the majority of the genetic variation in the core collection has been preserved in the mini core collection. Similar results were observed for mini core collections for chickpea (Cicer arietinum L., Upadhyaya and Ortiz, 2001) and peanut (Upadhyaya et al., 2002) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

No studies have been conducted to measure how ef-

fective a mini core and a two-stage screening approach would be to identify valuable genes in a core collection. We used data on disease resistancs for accessions in the core collection to retrospectively determine how effective the use of a mini core would have been in identifying sources of resistance in the core collection. Use of the core of the core collection would have improved the efficiency of identifying sources of resistance to leaf spot in the core collection (Table 2). The use of this two-stage screening approach would have required screening 54% of the core collection, but would have identified 90% (131 of 146) of the leaf spot-resistant accessions in the core collection. It has previously been shown that the core collection could be used to improve the efficiency of identifying resistance to leaf spot in the entire germplasm (Holbrook and Anderson, 1995). Since the core of the core approach would have identified nine out of 10 resistant accession in the core collection, it appears that this mini core collection could have been used to greatly improve the efficiency of identifying genes for resistance to leaf spot in the entire germplasm collection.

The core of the core approach could also have been used to improve the efficiency of identifying resistance to tomato spotted wilt virus in the core collection (Table 3). Using this approach only 25% of the core collection would have needed to be evaluated to identify 72% of the resistant accessions in the core collection.

Use of this mini core collection would not have significantly improved the efficiency of identifying resistance to the peanut root-knot nematode in the core collection (Table 4). This was unexpected since we had previously demonstrated that some geographical areas are rich sources of resistance to the peanut root-knot nematode (Holbrook et al., 2000a; Holbrook and Isleib, 2001) and shown that the clusters used to develop the core collection improved the efficiency of identifying nematode resistance in the entire collection (Holbrook et al., 2000b). An examination of the mean data for accessions showed similar grouping in the clusters used to select this core of the core collection. Cluster 24 consisted primarily of accessions from China, and 42% (5 of 12) of the accessions in that cluster were resistant to the peanut rootknot nematode. However, random sampling chose a susceptible accession as the indicator accession for this cluster. If a resistant accession had been chosen, then the chi-square analysis in Table 4 would have been significant. An adjustment was made in the core of the core to include representation for this group of accessions.

Use of the core of the core collection would not have improved the efficiency of identifying resistance to pre-

Table 2. Comparison of success rates for identifying resistance to leaf spot in various subsets of the U.S. core collection of peanut.

Subset	Resistant	Susceptible	Total	Success rate†	χ ²
Clusters with resistant indicator accessions‡	131	247	378	34.7	92.5**
Clusters with susceptible indicator accessions	15	306	321	4.7	
Total	146	553	699	20.9	

^{**} Significant at $P \leq 0.01$

 $[\]dagger$ Success rate = (no. of resistant accessions identified/total no. of accessions screened) \times 100.

[‡] Indicator accession is a core of core accession that indicates the expected response of the germplasm from the core collection that was included in the cluster from which the indicator was selected.

Table 3. Comparison of success rates for identifying resistance to tomato spotted wilt virus in various subsets of the U.S. core collection of peanut.

Subset	Resistant	Susceptible	Total	Success rate†	χ^2
Clusters with resistant indicator accessions‡	50	144	194	25.8	90.4**
Clusters with susceptible indicator accessions	19	557	576	3.3	
Total	69	701	770	9.0	

^{**} Significant at $P \leq 0.01$

Table 4. Comparison of success rates for identifying resistance to *Meloidogyne arenaria* in various subsets of the U.S. core collection of peanut.

Subset	Resistant	Susceptible	Total	Success rate†	χ²
Clusters with resistant indicator accessions‡	17	120	137	12.4	3.7NS
Clusters with susceptible indicator accessions	41	541	582	7.0	
Total	58	661	719	8.1	

NS. Not significant at $P \leq 0.05$

 \dagger Success rate = (no. of resistant accessions identified/total no. of accessions screened) \times 100.

Table 5. Comparison of success rates for identifying resistance to preharvest aflatoxin contamination in various subsets of the U.S. core collection of peanut.

Subset	Resistant	Susceptible	Total	Success rate†	χ^2
Clusters with resistant indicator accessions:	22	91	113	19.5	2.9NS
Clusters with susceptible indicator accessions	48	346	394	12.2	
Total	70	437	507	13.8	

NS, Not significant at $P \leq 0.05$

 \dagger Success rate = (no. of resistant accessions identified/total no. of accessions screened) \times 100.

harvest aflatoxin contamination (Table 5). This was not surprising since aflatoxin contamination of peanut is an extremely variable characteristic (Holbrook et al., 1994), and testing in multiple environments is necessary to improve accuracy. In addition, *Aspergillus* is a saprophyte on peanut, so a true pathogen–host relationship is not established. Reduced preharvest aflatoxin contamination appears to be a very rare trait in peanut, and a core collection approach will not improve the efficiency of identifying very rare genes in germplasm collections.

To maximize the usefulness of this core of the core collection, the relationship between individual accessions and the clustering procedure used to develop this subset of germplasm must be presented in two table formats. The first format lists the accessions in numerical order so that the cluster designation for individual accessions can be rapidly determined. The second format lists accessions by clusters so that all accessions within a cluster can be rapidly determined. Electronic copies of both tables are available, free of charge, from the corresponding author. This information will also be entered into GRIN.

In summary, this research has resulted in the development of a core of the core collection for peanut germplasm. This subset of germplasm should result in more effective and efficient utilization of peanut germplasm. This core of the core collection should be revised periodically as additional information and accessions are obtained. A core of the core approach should be very

useful for traits which are difficult and/or expensive to measure.

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